# ORGANOCHLORINE PESTICIDE AND HEAVY METAL CONTAMINATION OF AMERICAN CROCODILE (_CROCODYLUS_ _ACUTUS_) EGGS

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**Title:** **Organochlorine pesticide and heavy metal contamination of American Crocodile (Crocodylus acutus) eggs**

**Abstract:** Four eggs collected from two nests of the American crocodile (Crocodylus acutus), an endangered species occurring in southern Florida, were analyzed for organochlorine pesticide residues, mercury and lead content. A variety of organochlorine compounds, including polychlorinated biphenyls, were found, but concentrations were low when compared to other species. Lead was not detected in any of the eggs and mercury was only detectable in two of the four eggs. It does not appear that any of these contaminants, with the possible exception of PCBs, were responsible for non-viable eggs.

**Key Words:** crocodile, organochlorine, polychlorinated biphenyls, mercury, lead, pesticides, heavy metals

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## INTRODUCTION

The American crocodile (Crocodylus acutus), listed as an endangered species by the State of Florida, the U. S. Fish and Wildlife Service and the International Union for the Conservation of Nature, may be found in estuarine swamps from southern Florida southward to northern South America. Most of the U. S. population of some three hundred animals inhabits the Everglades National Park, Florida (Mazzotti 1989), but two other somewhat disjunct populations are found in the cooling water canals at Turkey Point nuclear power plant in Dade County and at Crocodile Lake National Wildlife Refuge, Key Largo, Monroe County.

Whereas nearly three-fourths of 104 nests examined by Mazzotti (1989) produced at least one hatchling, less than half (43%) of the eggs laid during the 12-year period from 1970 to 1982 produced hatchlings. As predation and embryo death due to flooding or desiccation only accounted for approximately 26% of the losses (Mazzotti 1989), several researchers (Stoneburner and Kushlan 1984, Hall et al. 1979, Ogden et al. 1974) began to suspect that contaminants, particularly pesticides and heavy metals, might account for the inviability of many of the remaining eggs.

This report provides some additional data regarding organochlorine pesticide residue and heavy metal concentrations in crocodile eggs.

## MATERIALS AND METHODS

Eggs were refrigerated after collection, and transferred to the U. S. Fish and Wildlife Service laboratory in Vero Beach where they were weighed and measured. Volume was calculated from the measurements as all eggs were broken when collected. After measurement, eggs were wrapped in two thicknesses of aluminum foil, placed in individual acid-cleaned glass jars sealed with teflon-lined lids, and frozen awaiting shipment to the analytical laboratory.

Analyses for organochlorine pesticides and polychlorinated biphenyls (PCBs) were performed by the Mississippi State Chemical Laboratory, Mississippi State University. Ten gram tissue samples were thoroughly mixed with anhydrous sodium sulfate and soxhlet extracted with hexane. After concentration by rotary evaporation, the extract was concentrated to dryness for lipid determination. The lipid sample was dissolved in petroleum ether and extracted four times with acetonitrile saturated with petroleum ether. Residues were then partitioned into petroleum ether, washed, concentrated and transferred to a glass chromatographic column containing 20 g of florisil. The column was eluted with 200 ml of 6% diethyl ether/94% petroleum ether (Fraction I) followed by 200 ml 15% diethyl ether/85% petroleum ether (Fraction II). Quantification of residues and PCBs from the two fractions was performed by electron capture gas chromatography. Recoveries ranged from 63% (hexachlorobenzene) to 100% (heptochlor epoxide and p,p'-DDD). For those residues reported herein, recoveries ranged from 85 to 100%. Results were not adjusted for recovery.

Aliquots of each sample were submitted to Research Triangle Institute, Research Triangle Park, NC, for lead and mercury analysis. After homogenization, tissue samples were freeze-dried to determine moisture content. Digestion of a 0.25 to 0.5 g aliquot of freeze-dried tissue was accomplished by heating the sample in a capped 120 ml Teflon vessel in the presence of 5 ml of Baker Instra-Analyzed nitric acid for three minutes at 120 watts, three minutes at 300 watts, and 15 minutes at 450 watts. The residue was then diluted to 50 ml with laboratory pure water. Metal content was determined by graphite furnace atomic absorption. A Perkins-Elmer Zeeman 3030 atomic absorption spectrophotometer with an HGA-600 graphite furnace and an AS-60 autosampler was used for the measurements. Detection limits were less than 0.1 and 0.2 ppm dry weight for mercury and lead, respectively.

All means reported herein are geometric means unless otherwise designated.

## RESULTS AND DISCUSSION

Physical characteristics of the four eggs examined are presented in Table 1. Organochlorine pesticide residue levels (Table 2), including polychlorinated biphenyls (PCBs), were generally similar to, or slightly lower than, those reported by Hall et al. (1979). Only heptachlor epoxide (HE) and PCB concentrations were higher. But, whereas HE was only slightly higher (mean = 0.027 ppm wet weight in this study vs. 0.019 (n=19); Hall et al. 1979), PCB levels were considerably higher. The mean PCB concentration for the four eggs in this study was 1.37 ppm wet weight whereas Hall et al. (1979) only found approximately 0.40 ppm (n=23). Ogden et al. (1974) did not detect any PCBs in five eggs collected from Everglades National Park in 1972. It would thus appear that PCB levels in eggs of the American crocodile are either greater in eggs from Crocodile Lake than in those from Everglades National Park, or that PCB levels are increasing over time. In addition, although Hall et al. (1979) detected dieldrin, cis-chlordane and p,p'-DDT in eggs collected in 1977-1978, we did not detect any of these residues.

Heavy metal concentrations in the four eggs collected at Crocodile Lake (Table 3) were less than those reported by Stoneburner and Kushlan (1984) and by Ogden et al. (1974). In this study, lead concentrations were below the detection limit (≈

Table 1. Weight, volume, percent moisture, and percent lipid of four inviable crocodile eggs from Crocodile Lake National Wildlife Refuge.

| | Parameter | | | |
|---|---|---|---|---|
| Sample ID | Weight (g) | Volume (ml) | Moisture (%) | Lipid (%) |
| CL9101 | 58.5 | 71.7 | 67.5 | 12.0 |
| CL9102 | 60.3 | 70.0 | 70.0 | 10.3 |
| CL9103 | 67.8 | 74.5 | 74.0 | 9.11 |
| CL9104 | 71.0 | 74.8 | 76.0 | 8.79 |

Table 2. Organochlorine residues in crocodile eggs from Crocodile Lake National Wildlife. Only those residues actually detected are presented. Concentrations expressed as ppm wet weight.

| | Sample ID | | | |
|---|---|---|---|---|
| Compound | CL9101 | CL9102 | CL9103 | CL9104 |
| oxychlordane | 0.01 | 0.02 | 0.02 | 0.02 |
| heptachlor epoxide | 0.02 | 0.03 | 0.03 | 0.03 |
| t-nonachlor | ND[a] | 0.01 | 0.02 | 0.03 |
| cis-nonachlor | 0.02 | 0.02 | 0.03 | 0.03 |
| p,p'-DDD | 0.01 | 0.01 | 0.02 | 0.02 |
| p,p'-DDE | 0.46[b] | 0.45 | 0.51 | 0.44 |
| Mirex | 0.01 | 0.01 | 0.01 | 0.01 |
| PCBs (total) | 1.5[b] | 1.4 | 1.3 | 1.3 |

[a] ND = not detected.
[b] Confirmed by GC/Mass Spectrometry

Table 3. Lead and mercury concentrations in crocodile eggs from Crocodile Lake National Wildlife. Concentrations expressed as ppm wet weight.

| | Sample ID | | | |
|---|---|---|---|---|
| Element | CL9101 | CL9102 | CL9103 | CL9104 |
| Lead | <0.078 | <0.055 | <0.056 | <0.051 |
| Mercury | 0.059 | 0.031 | <0.028 | <0.025 |

0.05 ppm wet weight). Stoneburner and Kushlan (1984) and Ogden et al. (1974) reported mean lead concentrations of 0.64 and 0.34 ppm wet weight, respectively. Mercury levels in this study ranged from <0.025 to 0.059 ppm wet weight (Table 3) as compared to 0.13 and 0.09 ppm wet weight reported by Stoneburner and Kushlan (1984) and Ogden et al. (1974).

We did not find any information in the literature relating to the sensitivity of crocodilians to either mercury or lead, nor were we able to find any data regarding the impact of organochlorine residues to these animals. However, mercury concentrations in the two eggs in which the element was detected were well below the level (0.79-2.0 ppm) noted by Eisler (1987) as being linked to impaired reproduction in birds. Likewise, with the possible exception of p,p'-DDE and total PCBs, organochlorine levels were barely above the detection limit of 0.01 ppm and thus, not likely to pose any significant impact to the crocodile.

In the case of p,p'-DDE, the mean concentration (= 0.46 ppm wet weight) was less than levels noted in eggs of several species of fish-eating birds (range: 0.9 to 37.0 ppm; Vermeer and Reynolds 1970), and several orders of magnitude less than that reported to cause reproductive impairment in the European sparrowhawk (Accipiter nisus; ≈ 154 ppm in egg lipids) reported by Newton et al. (1979). Eisler (1986) reported that PCB residues in eggs from successful nests of the American bald eagle (Haliaeetus leucocephalus) were significantly lower than in eggs from unsuccessful nests (1.3 ppm wet weight vs. 7.2 ppm). Bryan et al. (1987), however, pointed out that some PCB congeners are much more toxic than others. Therefore, although PCB levels in those crocodile eggs examined were similar to levels in the most successful eagle nests, it is possible that one or more of the more toxic PCB congeners might have an impact on recruitment in crocodile populations in Florida. It is highly recommended that future studies address PCB contamination in the eggs of all Florida crocodilians.

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